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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Application of Brian W. Ward  
Serial No. : 09/610,935  
Filed : July 6, 2000  
Confirmation No. 5148

Art Unit 1634

For TARGET REAGENTS THAT ENHANCE REACTION-PRODUCT ANALYSIS

Commissioner for Patents  
P.O. Box 1450  
Alexandria VA 22313-1450

**DECLARATION OF PRIOR INVENTION IN THE UNITED STATES OR IN A NAFTA  
OR WTO MEMBER COUNTRY TO OVERCOME CITED PATENT OR PUBLICATION  
(37 C.F.R. § 1.131)**

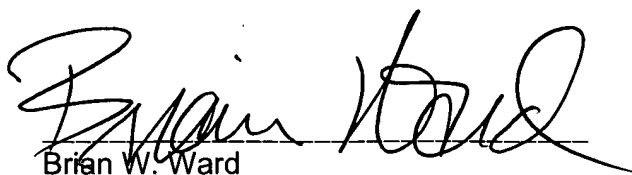
I, Brian W. Ward declare as follows:

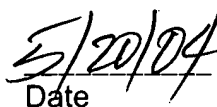
1. I am an inventor of the subject matter claimed in the above-entitled United States patent application.
2. I am submitting this Declaration to establish completion of our invention in the U.S. before December 7, 1998, the filing date of U.S. Patent No. 6,153, 412.
3. The invention claimed in this application was reduced to practice in the United States before December 7, 1998.
4. Facts in support of this Declaration are attached hereto as Exhibit A. Exhibit A is a true and correct copy of experimental note book pages, data sheets, and graphs, each Bates-stamped as exhibit pages 00001-00038. Dates have been redacted from these pages but all such dates are before December 7, 1998.
5. The matter contained in Exhibit A is that upon which Figures 11, 12A, 12B, 13, 15, 16, and Example 1 of this application are based. Specifically, page 00003

corresponds to Figure 11; page 00008 corresponds to Figure 12A; page 00011 corresponds to Figure 12B; page 00012 corresponds to Figure 16; page 00029 corresponds to Figure 13; and page 00035 corresponds to Figure 15 of this application.

6. The matter contained in Exhibit A reflects embodiments of the claims of the present application. An aqueous reagent containing Taq DNA polymerase, anionic tracer dye, and a solute to increase the physical density, but not containing primer or nucleic acid polymer template, is referenced on page 0021 and 0038. Preparation of such reagent with acid red 1 (AR1) and acid violet 5 (AV5) anionic tracer dye is referenced throughout the provided pages. An 85/15 proportion of AV1/AV5 is referenced on pages 00014, 00016, 00017, 00020, 00021, A solute (specifically, glycerol) that increases the physical density of the reagent is referenced on pages 00001, 00012, 00013, and 00038. A reagent with Taq DNA polymerase, anionic tracer dye, solute, no primer or template, and a density of at least  $1.01 \text{ g/cm}^3$  (and specifically,  $1.14 \text{ g/cm}^3$ ) is referenced on page 0038. Optical densities between 5 and 500 in the reagent are referenced on pages 00016, 00021, and 00038. Taq DNA polymerase concentrations between 0.033 and 10 units/ $\mu\text{l}$  of reagent are referenced on pages 00015, 00021, 00026, 00034, and 00038. As such, Exhibit A evidences our reduction to practice of the invention claimed in the application prior to December 7, 1998.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

  
Brian W. Ward

  
Date

50% glyc	50% gly	AV5	H <sub>2</sub> O
% gly		9 mg/ml	
5	10	25	65
4.5	9	↓	66
4	8		67
3.5	7		68
3	6		69
2.5	5		70

load OK to 1.4% or lower

1ml 2.5% glycerol 1X PCR

50  $\mu$ l 50% glyc, 100  $\mu$ l 10X PCR, 850 H<sub>2</sub>O

in PCR ~ 0.8 mg/ml  $\rightarrow$  Enz 16 mg/ml

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Date

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*[Signature]*

Date

To Page No. \_\_\_\_\_

PCR Yield

36 reactions - <sup>20</sup>~~40~~  $\mu$ l ea -

20  $\mu$ l dge, 20  $\mu$ l ~~EX~~ PCR mix

PCR MIX - (<sup>200</sup>~~800~~  $\mu$ l) 10X - <sup>80</sup>~~400~~  $\mu$ l

10mM dNTP's - <sup>8</sup>~~16~~  $\mu$ l,  $\alpha$  <sup>2.5</sup>~~5~~  $\mu$ l  $^{32}$ P dCTP

PMRS <sup>40</sup>~~80~~  $\mu$ l ea, Temp <sup>40</sup>~~80~~  $\mu$ l, Tacy 40  
 $H_2O$  149.5

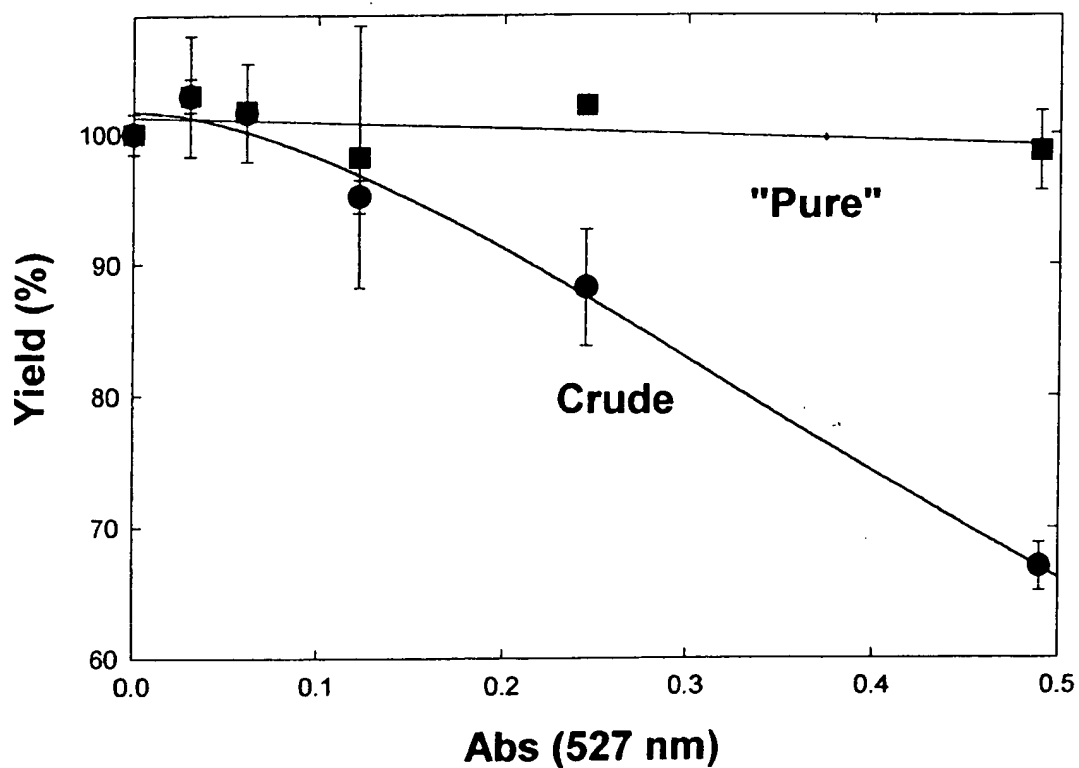
1	7	12
A AV5	0 AV	0
B AR1	0 AR5	0
C AV/AR	0 AV/AR	0

AV5, AR1 + <sup>10</sup>~~32~~  $\mu$ l  $H_2O$   $\rightarrow A_{max} = 100$

AV/AR 8 ea + 4  $H_2O$   $\rightarrow Total = 100$

*Finan David*

**PCR yield of crude vs. pure (desalted)  
Acid Violet 5 containing reactions.  
Template/primer= lambda 500mer,  
20 cycles at 94/55/72°C, 1 min each.  
Abs=0.5 approx. 0.5 mg/ml of crude dye.**



Purified "AV5"

USER: 1 ID:GONEHEAD PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N R6232:N  
 H#: 0 ADD:N BCF:N RCM:N  
 CHANNEL 1-LL: 0 UL:1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000  
 HALF LIFE(DAYS):N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	271360.00	1.72	0.05	0.22	
2	**	2	363120.00	1.48	0.05	0.60	
3	**	3	374700.00	1.46	0.05	0.97	
4	**	4	414120.00	1.39	0.05	1.35	
5	**	5	421720.00	1.38	0.05	1.72	
6	**	6	404520.00	1.41	0.05	2.10	
7	**	7	260940.00	1.75	0.05	2.47	
8	**	8	338120.00	1.54	0.05	2.84	
9	**	9	381780.00	1.45	0.05	3.21	
10	**	10	393240.00	1.43	0.05	3.58	
11	**	11	395820.00	1.42	0.05	3.96	
12	**	12	393980.00	1.42	0.05	4.33	
13	**	1	400560.00	1.41	0.05	4.80	
14	**	2	404860.00	1.41	0.05	5.17	
15	**	3	418380.00	1.38	0.05	5.55	
16	**	4	404260.00	1.41	0.05	5.92	
17	**	5	405360.00	1.40	0.05	6.30	
18	**	6	393660.00	1.43	0.05	6.67	
19	**	7	383680.00	1.44	0.05	7.05	
20	**	8	406880.00	1.40	0.05	7.42	
21	**	9	362080.00	1.49	0.05	7.80	
22	**	10	403980.00	1.41	0.05	8.17	
23	**	11	412540.00	1.39	0.05	8.55	
24	**	12	148290.00	1.64	0.10	8.97	

0.98/2 Abs

Bulk

0.98/2 Abs

Pure

From Page No. \_\_\_\_\_

Mg AR1 / AVE

1A → 12

Mg Dyes.

2A → 12

10<sup>4</sup> Dyes + MgCl<sub>2</sub>Mg Dyes - 10<sup>4</sup> Dye @ A<sub>max</sub> = 100, 10<sup>4</sup> 10<sup>4</sup> 10<sup>4</sup>MgCl<sub>2</sub> + H<sub>2</sub>O[MgCl<sub>2</sub>]1 → 6 [MgCl<sub>2</sub>] + 1X PCR4X MgCl<sub>2</sub> 40, 32, 24,  
16, 8 mM100<sup>4</sup> MgCl<sub>2</sub> H<sub>2</sub>O 96, 96.8, 97.6, 98.4, 99.11M MgCl<sub>2</sub> 4 3.2 2.4 1.6 0.8

To Page 1

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TTT

Project No. \_\_\_\_\_

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From Page No. \_\_\_\_\_

Zn, Ca & Mg AR $\pi$

2X mix 225  $\mu$ l,

10X 45  $\mu$ l, dNTP's 4.5  $\mu$ l

Primer/Temp 22.5  $^{\circ}$ C

$\alpha^{32}$ P dCTP 4  $\mu$ l Tag 22.5

H<sub>2</sub>O 83.6

~~PI EDTA Time course~~

~~comp<sup>2</sup> (cutting)~~

Bulk  $\rightarrow$

	10X	13.2	13.2
	PA20	1	1
	Origo	25.1	<del>25.1</del> 13.2
	H <sub>2</sub> O	80.7	92.6

To Page No \_\_\_\_\_

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Project No. \_\_\_\_\_

TITLE \_\_\_\_\_ Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

Zn, Ca { Mg AR  $\pi$

2X mix 225  $\mu$ l,

10X 45  $\mu$ l, dNTP's 4.5  $\mu$ l

Prim/Temp 22.5  $^{\circ}$ C

$\alpha^{32}$ P dCTP 4  $\mu$ l Tag 22.5

H<sub>2</sub>O 83.6

~~32P EDTA Time course~~

~~comp 2 (cutting)~~

~~Back~~

~~→ PA20~~

~~Oligo~~

~~H<sub>2</sub>O~~

~~T<sub>20</sub>~~

~~13.2~~

~~1~~

~~25.1~~

~~80.7~~

~~13.2~~

~~1~~

~~25.1 13.2~~

~~92.6~~

To Page No. \_\_\_\_\_

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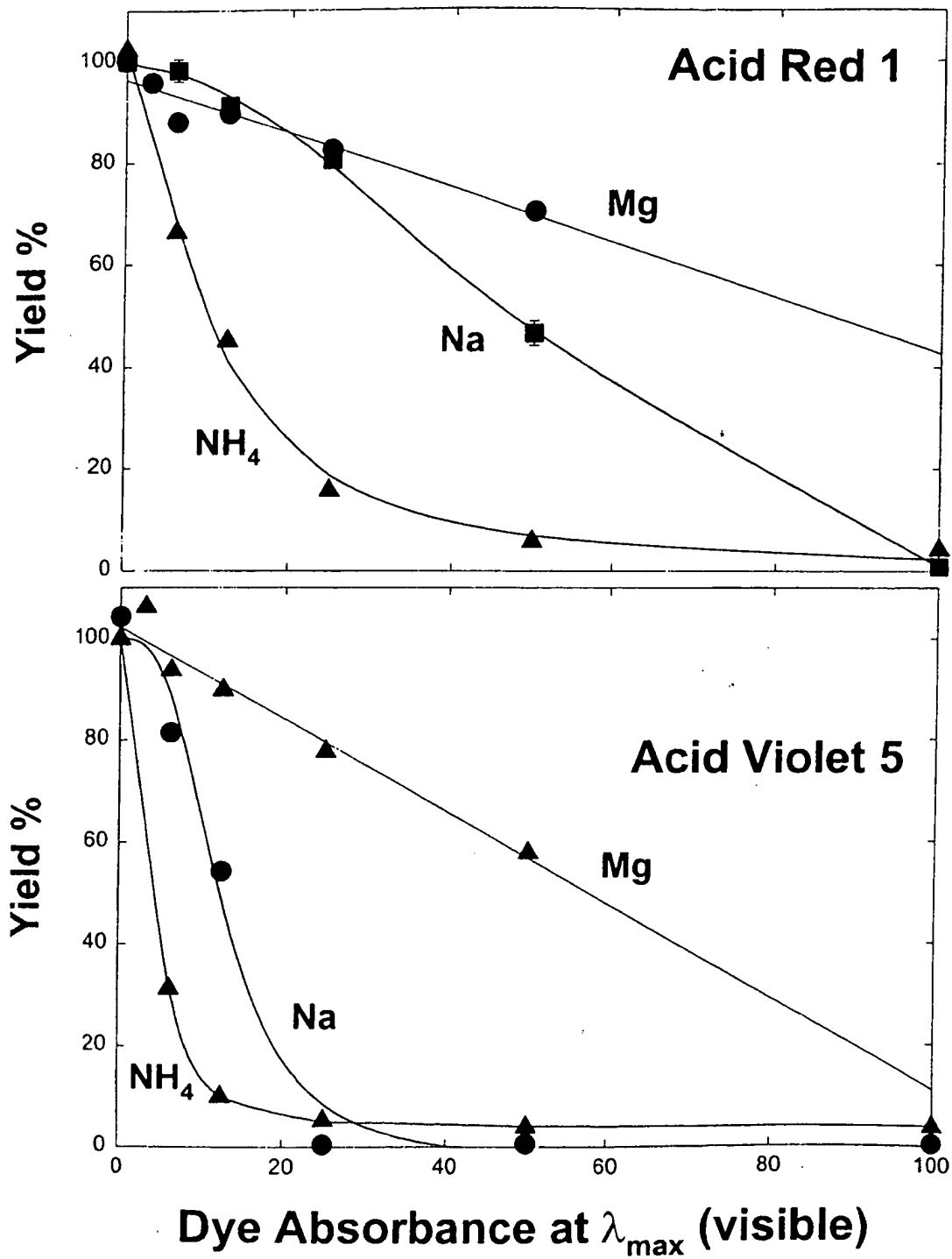
Date

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00007

*Simon H. H. Q.*

## Counterion dependence of PCR yield



USER: 1 ID: BONEHEAD PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
 HH: 0 ADD: N GCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALD: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000  
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	1284.00	5.58	1.00	1.18	70
2	**	2	904.00	6.65	1.00	2.52	8
3	**	3	967.00	6.43	1.00	3.87	6 + MgCl <sub>2</sub>
4	**	4	1076.00	6.10	1.00	5.21	4 mM
5	**	5	1863.00	4.63	1.00	6.55	2
6	**	6	16424.62	1.94	0.65	7.53	0
7	**	7	474320.00	1.30	0.05	7.90	50
8	**	8	555580.00	1.20	0.05	8.28	25
9	**	9	604140.00	1.15	0.05	8.65	12.5
10	**	10	593400.00	1.16	0.05	9.03	4.25
11	**	11	646100.00	1.11	0.05	9.40	3.125
12	**	12	674380.00	1.09	0.05	9.78	0
13	**	1	1531.00	5.11	1.00	11.12	50
14	**	2	1454.00	5.25	1.00	12.52	25
15	**	3	1937.00	4.54	1.00	13.81	12.5
16	**	4	939.00	6.53	1.00	15.20	4.25
17	**	5	1297.00	5.55	1.00	16.54	3.125
18	**	6	1071.00	6.11	1.00	17.88	0
19	**	7	386520.00	1.44	0.05	18.26	50
20	**	8	520720.00	1.24	0.05	18.63	25
21	**	9	601440.00	1.15	0.05	19.01	12.5
22	**	10	627540.00	1.13	0.05	19.38	4.25
23	**	11	711500.00	1.06	0.05	19.77	3.125
24	**	12	669760.00	1.09	0.05	20.13	0

ARI-NH<sub>4</sub>

Mg ARI A<sub>532</sub>

+MgCl<sub>2</sub>  
AV5-NH<sub>4</sub>

A<sub>527</sub>  
AV5-Mg

JSER: 1 ID: BONEHEAD      PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N    RS232: N  
 ##: 0 ACC: N DCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CFM, UNKNOWN REPLICATES: 1    NORM FACTOR: 0 1.00000  
 HALF LIFE(DAYS): N

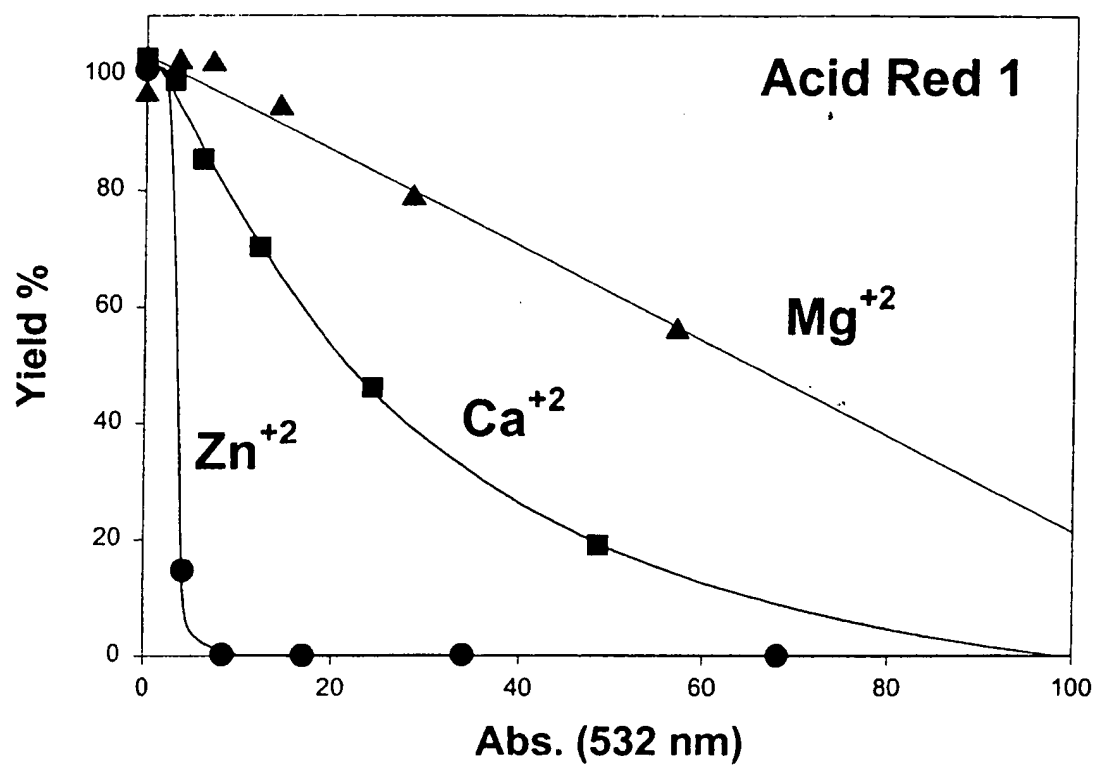
SAM	PDS	CH	CFM	2SIG%	TIME	EL TIME	EPR
1	**	1	662.00	7.77	1.00	1.18	
2	**	2	1306.00	5.53	1.00	2.50	
3	**	3	3133.00	3.57	1.00	3.84	
4	**	4	8838.00	2.13	1.00	5.18	
5	**	5	12747.50	1.98	0.80	6.33	
6	**	6	19934.54	1.91	0.55	7.21	
7	**	7	814.00	7.01	1.00	8.58	
8	**	8	780.00	7.16	1.00	9.88	
9	**	9	2486.00	4.01	1.00	11.23	
10	**	10	7301.00	2.34	1.00	12.56	
11	**	11	10994.74	1.96	0.95	13.61	
12	**	12	16646.15	1.92	0.65	14.83	
13	**	1	746.67	18.90	0.15	15.39	
14	**	2	1123.33	10.89	0.30	16.06	
15	**	3	888.89	10.00	0.45	16.80	
16	**	4	1795.00	6.09	0.60	17.83	
17	**	5	5274.74	2.83	0.95	19.12	
18	**	6	17731.67	1.74	0.60	20.07	
19	**	7	655.00	7.81	1.00	21.43	
20	**	8	670.00	7.73	1.00	22.77	
21	**	9	895.00	6.69	1.00	24.11	
22	**	10	1719.00	4.82	1.00	25.42	
23	**	11	5380.00	2.73	1.00	26.77	
24	**	12	17263.33	1.97	0.60	27.70	

ARI - NH<sub>4</sub>

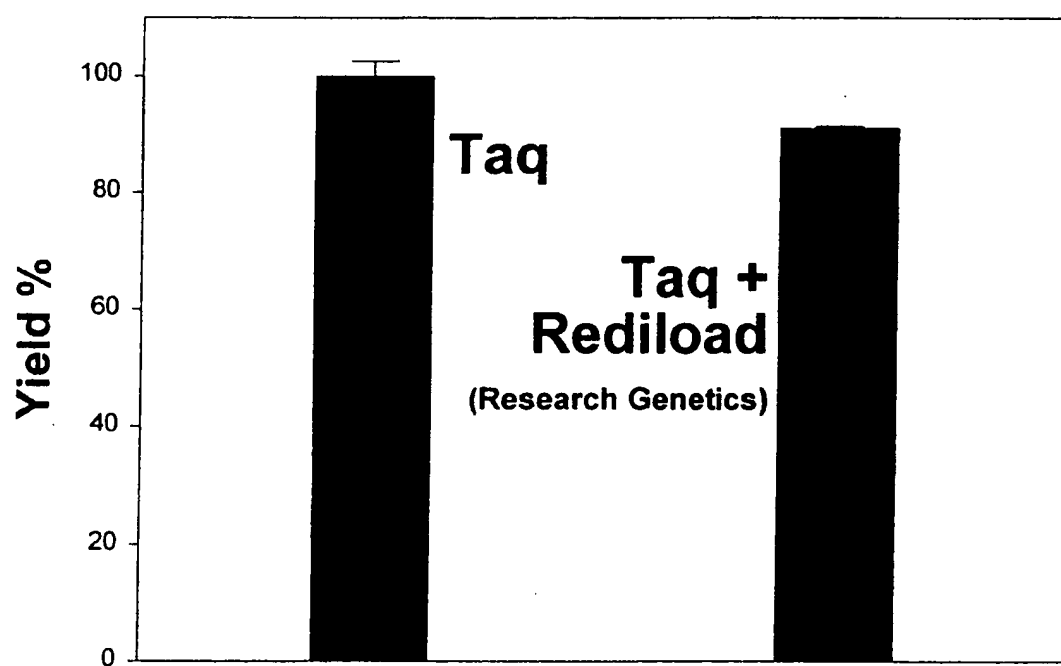
AV5 NH<sub>4</sub>

*Handwritten signature*

# Effect of divalent counter ion on PCR yield.



**Yield of  $\lambda$  500mer for Taq  
and Taq plus *Rediload***



USER: 1 ID: BONEHEAD PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
 H#: 0 ADD: N QCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALD: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000  
 HALF LIFE (DAYS): N

SAM	PBS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	153860.00	1.61	0.10	0.27	
2	**	2	370180.00	1.47	0.05	0.65	
3	**	3	563220.00	1.19	0.05	1.02	
4	**	4	684200.00	1.08	0.05	1.39	Ca
5	**	5	792840.00	1.00	0.05	1.77	
6	**	6	822840.00	0.99	0.05	2.15	
7	**	7	1180.00	5.82	1.00	3.49	
8	**	8	1840.00	4.66	1.00	4.81	
9	**	9	1136.00	5.93	1.00	6.13	Zn
10	**	10	1825.00	4.68	1.00	7.48	
11	**	11	118080.00	1.84	0.10	7.90	
12	**	12	607280.00	1.00	0.05	8.28	
13	**	1	448040.00	1.34	0.05	8.72	136.5
14	**	2	630220.00	1.13	0.05	9.09	67.9
15	**	3	754640.00	1.03	0.05	9.48	35.8
16	**	4	814480.00	0.99	0.05	9.85	16.43
17	**	5	817500.00	0.99	0.05	10.23	3.96
18	**	6	773140.00	1.02	0.05	10.62	0
19	**	7	727080.00	1.05	0.05	10.99	
20	**	8	728560.00	1.05	0.05	11.37	
21	**	9	732000.00	1.05	0.05	11.74	Redi Load.

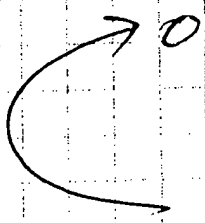


TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

85% AR1 / 15% AV5 titration

A <sub>TOT</sub>	2X	V 5/4 → A <sub>TOT</sub> = 500 (200 μl)	V Tag Strang Buffer
20	40	16	0
18	17.5	35	2
16	15	30	4
14	12.5	25	6
12	10	20	8
10	0	0	0



H<sub>2</sub>O - compare yield to 5 μl

NXNS - 24 NXNS 12 500mer,  
12 5000mer

~~2X as pg 84~~

2X (150 μl) 10X 30 μl, 10 μl dNTP's 3,  
Pms 6 μl ea Temp 15 μ,  
α<sup>32</sup>P dCTP 2 μl, Tag 3,  
85 H<sub>2</sub>O

To Page \_\_\_\_\_

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*[Signature]*

Date \_\_\_\_\_

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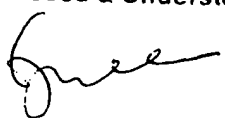
Recorded by

Date \_\_\_\_\_

From Page No. \_\_\_\_\_

2X (137.5  $\mu$ l) 10X 27.5  $\mu$ l, pmrs 5.5  $\mu$ l  
2 14  $\mu$ l, dNTP's 2.75  $\mu$ l,  
<sup>32</sup>P dCTP 1.5  $\mu$ l, ENZ 2.75  $\mu$ l  
5  $\mu$ l/ $\mu$ l  
H<sub>2</sub>O 78

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Acid Red, AR1/AV5 mixes

24 rxns. 12 rxn ea pmr 20,  
1

~~AR1/AV5~~

70 AR1

100  
82.5  
65  
0

Pmr / Temp  $\lambda = \text{temp.}$

Pmrs @ 50X

2X - (275  $\mu$ l ea) 27.5  
55  $\mu$ l 10X  
pmrs 5.5  $\mu$ l ea,  $\lambda$  27.5  $\mu$ l 14  
dNTP's 2.75, 32P dCTP 1.5  
E112 5.5  $\mu$ l 3  $\mu$ l  
H<sub>2</sub>O 156.5 2.75 5.5  $\mu$ l Tq @ 5.

2X dyes. 4  $\mu$ l + 46  $\mu$ l H<sub>2</sub>O

0.8  $\mu$ l ~~500~~ A=500 per rxn -

$\frac{10}{\text{rxn}} \leftarrow \frac{48 \mu\text{l}}{50}$

Witnessed & Understood by me,

*[Signature]*

Date

Invented by

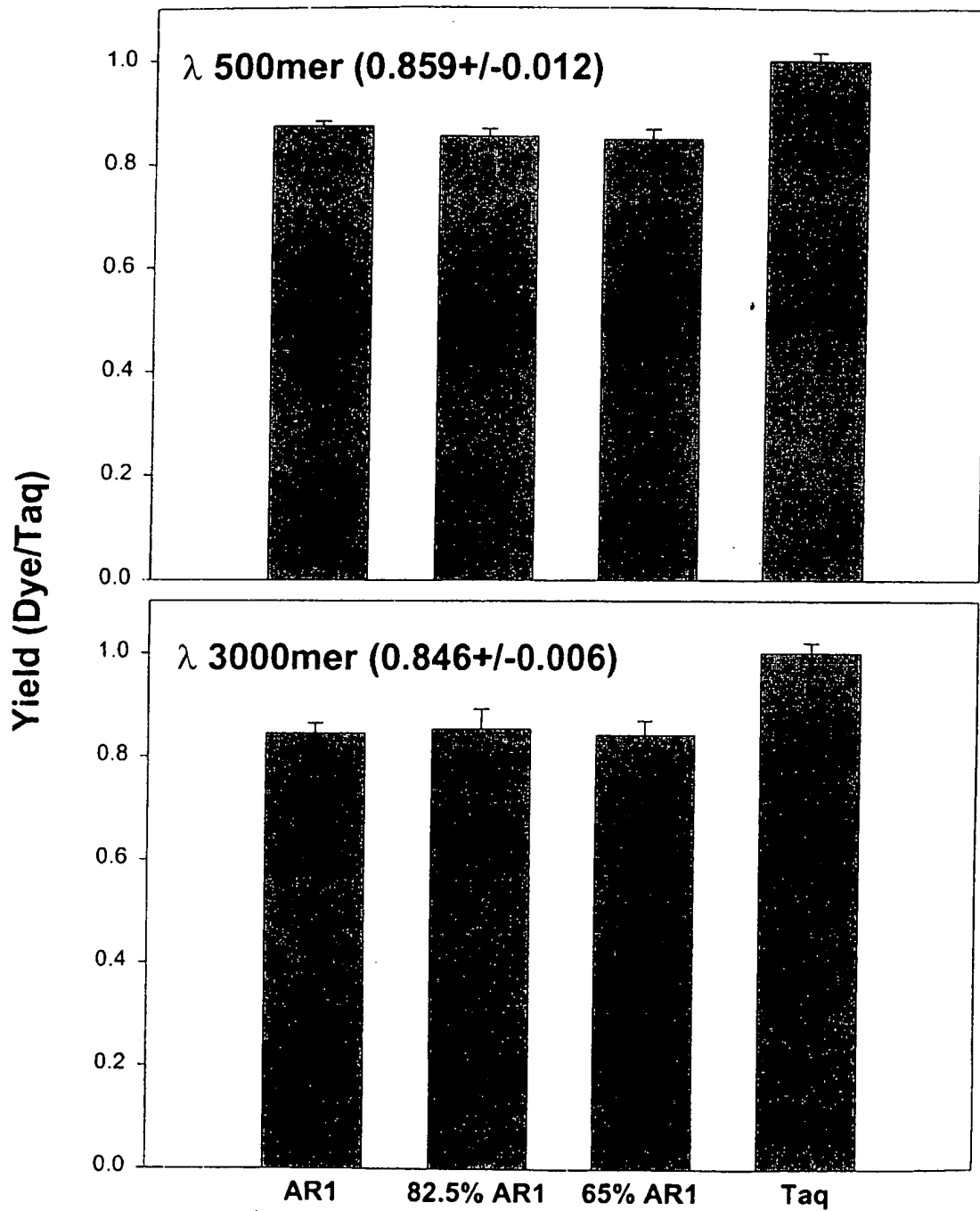
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Date

To Page

*Shan Patel*

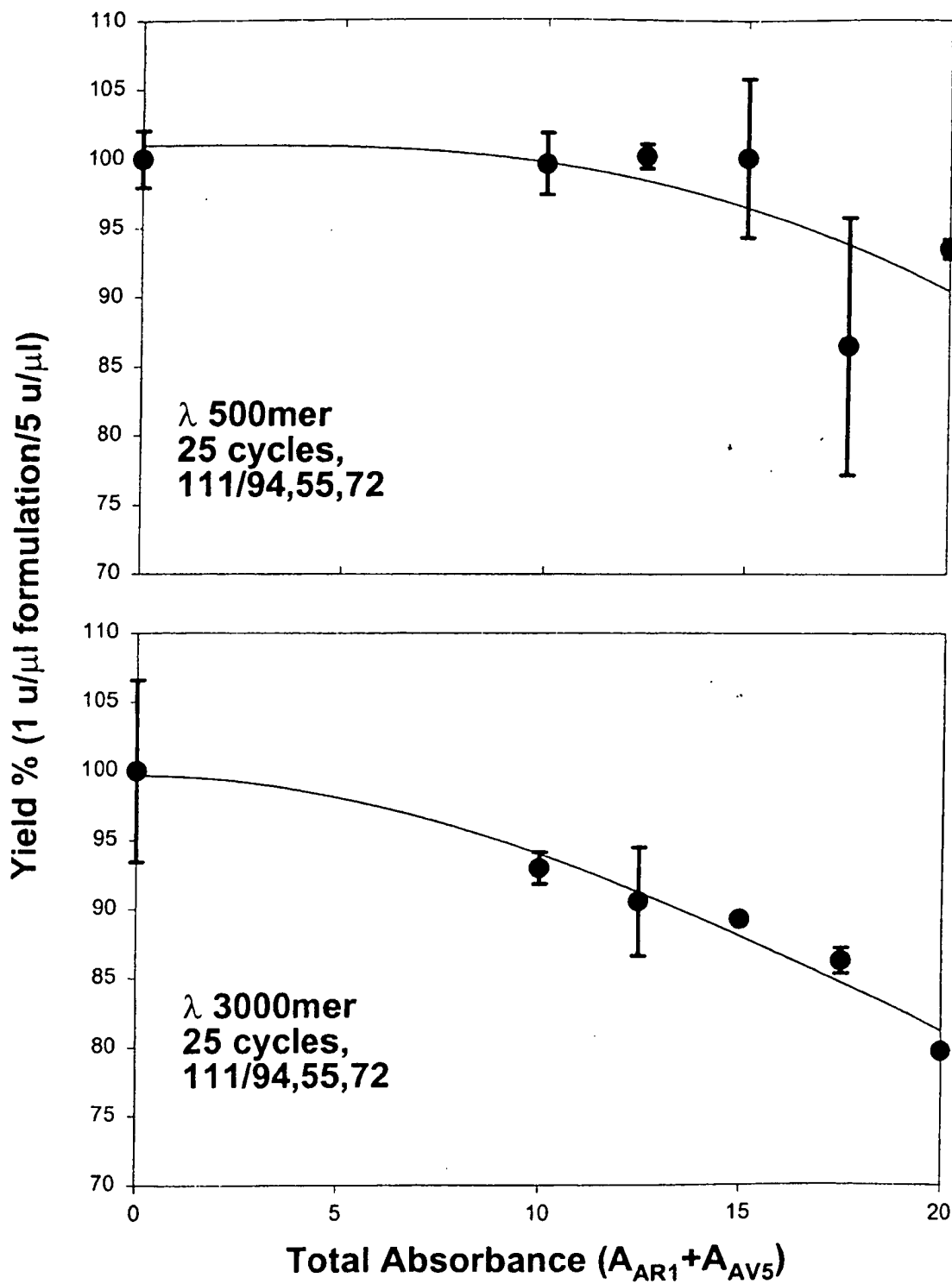
Acid Red 1, Acid Violet 5 mixes.  $A_{527} + A_{532} = 20$ .



USER: 1 ID: BONEHEAD PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
 H#: 0 AOC: N OCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALD: CPM, UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000  
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	AVI	ERR
1	**	1	262680.00	1.75	0.05	0.22	100	
2	**	2	250680.00	1.79	0.05	0.59	82.5	
3	**	3	253920.00	1.77	0.05	0.96	65	
4	**	4	304120.00	1.62	0.05	1.33	0	
5	**	5	262360.00	1.75	0.05	1.69	100	
6	**	6	258880.00	1.76	0.05	2.06	82.5	
7	**	7	248000.00	1.80	0.05	2.42	65	
8	**	8	295900.00	1.64	0.05	2.80	0	
9	**	9	257720.00	1.76	0.05	3.16		
10	**	10	256520.00	1.77	0.05	3.52		
11	**	11	259260.00	1.76	0.05	3.89		
12	**	12	296440.00	1.64	0.05	4.27		
13	**	1	187260.00	1.46	0.10	4.73		
14	**	2	188460.00	1.46	0.10	5.16		
15	**	3	188200.00	1.46	0.10	5.58		
16	**	4	224580.00	1.89	0.05	5.95		
17	**	5	180940.00	1.49	0.10	6.38		
18	**	6	178800.00	1.50	0.10	6.80		
19	**	7	177840.00	1.50	0.10	7.23		
20	**	8	219380.00	1.91	0.05	7.59		
21	**	9	189330.00	1.45	0.10	8.02		
22	**	10	195870.00	1.43	0.10	8.44		
23	**	11	188910.00	1.46	0.10	8.87		
24	**	12	216100.00	1.92	0.05	9.23		
25	**	1	452580.00	1.33	0.05	9.67		
26	**	2	453440.00	1.33	0.05	10.04		
27	**	3	448500.00	1.34	0.05	10.42		
28	**	4	422080.00	1.38	0.05	10.79		
29	**	5	264560.00	1.74	0.05	11.16		
30	**	6	264000.00	1.74	0.05	11.52		
31	**	7	276780.00	1.70	0.05	11.88		
32	**	8	260840.00	1.75	0.05	12.24		
33	**	9	286240.00	1.67	0.05	12.62		
34	**	10	281540.00	1.69	0.05	12.98		
35	**	11	295420.00	1.65	0.05	13.34		
36	**	12	271680.00	1.72	0.05	13.70		

*Shan V. Q.*  
**PCR yields as a function 85% AR1 concentration.**



85% AR1, 15% AV5

SER: 1 ID: BONEHEAD PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
 H#: 0 AQC: N QCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CFM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000  
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	A	ERR
1	**	1	592900.00	1.16	0.05	0.22	20.	
2	**	2	509080.00	1.25	0.05	0.61	17.5	
3	**	3	663160.00	1.10	0.05	0.98	15.	
4	**	4	641960.00	1.12	0.05	1.36	12.5	
5	**	5	644420.00	1.11	0.05	1.73	10.	
6	**	6	627980.00	1.13	0.05	2.11	0	
7	**	7	599180.00	1.16	0.05	2.48	20	
8	**	8	592960.00	1.16	0.05	2.86	17.5	
9	**	9	611580.00	1.14	0.05	3.23	15	
10	**	10	634020.00	1.12	0.05	3.61	12.5	
11	**	11	624500.00	1.13	0.05	3.98	10	
12	**	12	646420.00	1.11	0.05	4.36	20	
13	**	1	505300.00	1.26	0.05	4.82	17.5	
14	**	2	544660.00	1.21	0.05	5.20	15	
15	**	3	568020.00	1.19	0.05	5.57	12.5	
16	**	4	593700.00	1.16	0.05	5.95	10	
17	**	5	586300.00	1.17	0.05	6.32	0	
18	**	6	606820.00	1.15	0.05	6.70	20	
19	**	7	508140.00	1.25	0.05	7.07	17.5	
20	**	8	553040.00	1.20	0.05	7.45	15	
21	**	9	19154.54	1.95	0.55	8.33	12.5	
22	**	10	558160.00	1.20	0.05	8.71	10	
23	**	11	596720.00	1.16	0.05	9.08	0	
24	**	12	665980.00	1.10	0.05	9.46	0	

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0.85 ARI, <sup>0.15</sup> AVS @ A=500, 37.5

1 u/ml Tags.

Abs	500	37.5
ARI (615.6)	207	15.5
AVS (541)	41.6	31.2
STORAGE Buf	51.4	113.8

Witnessed &amp; Understood by me,



Date

Invented by

Recorded by

To Page

Date



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1500 &amp; 3000 mer (Mg) app

Dye/CTL

3/4 Dye

STAGE 20  $\mu$ l  
+ 180  $H_2O$ ~~20~~2X E mixes. 275  $\mu$ l ER.PMRS 11  $\mu$ l ER,  $\lambda$  13.5  $\mu$ l10 mM dATP'S 5.5  $\mu$ ld<sup>32</sup>P dATP 3  $\mu$ l, 5.5  $\mu$ l (5  $\mu$ l)225.5  $H_2O$  $\lambda$  1 (anchor)  
 $\lambda$  (1500)  
 $\lambda$  (3000)

1	2	3	4	5	6	7	8	9	10	11
15	12.5	10	8	7	6	5	4	3	2	1

10X [Mg]

10X + Mg

10X - Mg

Witnessed &amp; Understood by me,

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Date

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White      ~~2  $\mu$ l~~      10  $\mu$ l 2X      10  $\mu$ l 2X  
                  ~~8  $\mu$ l~~      10X      H<sub>2</sub>O      Buffer  
                  10  $\mu$ l      2X      Enz mix      } x 4

Red 15      ~~2  $\mu$ l~~      ~~1 mM Mg 10X~~  
                  10  $\mu$ l      ~~dye~~  
                  2X      10  $\mu$ l      2X      Enz mix      } x 4

Red 25      2  $\mu$ l      8 mM Mg 10X  
                  8  $\mu$ l      Dye  
                  10  $\mu$ l      2X      Enz mix      } x 4

2X 500 mer (135  $\mu$ l)      2.7  $\mu$ l Tag.      5.4  $\mu$ l

13.5  $\lambda$       2.7 dATP's

2  $\mu$ l <sup>32P</sup> dATP      103.3 H<sub>2</sub>O

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

Invented by

Date

Recorded by

00023

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Red Tag "opt" field

1. White @ 5 u/ $\mu$ l2. Red 15 (100  $\mu$ l)~~10  $\mu$ l 5/4 Dye~~~~70  $\mu$ l H<sub>2</sub>O~~

2x

5/4 Dye 8  $\mu$ l Dye10X + Mg 14.67  $\mu$ l10X - Mg 5.33  $\mu$ lH<sub>2</sub>O 72  $\mu$ l1.1 ml  
MgCl<sub>2</sub>3. Red 25 (100  $\mu$ l)

5/4 Dye 13.33

50% Ery 2.67

10X + Mg 10.67

10X - Mg 9.33

0.8 ml  
MgCl<sub>2</sub>

Witnessed &amp; Understood by me,

*Gu*

Date

Invented by

Recorded by

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Date

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10x 2x  
~~5x~~ 5x dge  
2x 10x

0.16	0.4	0	1	2	3	4	5	6	7	8	9	10	11	12
4	2.67	0	15	13	11	9	8	7	6	5	4	3	2	0
16	17.33	20												

(25x) 10x  
+ mg 20 17.33 14.67 12 10.67 9.33 8 6.67 5.33 4 2.67 0  
- mg 0 2.67 5.33 8 9.33 10.67 12 13.33 14.67 16 17.33 20

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*Green*

Date

Invented by

Recorded by

*Eric Wood*

Date

From Page No. \_\_\_\_\_

[Mg] app -

	1	2	3	4	5	6	7	8	9	10	11	12
[Mg]	1.5	1.3	1.1	0.9	0.7	0.5	0.3	0.2	0			
	1.5	1.3	1.1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	
				0.7	0.6	0.5	0.4	0.3	0.2	0.1	0	

10X

10X Buffers

~~200~~ 500  $\mu$ l 1003 2.6 2.2 1.8 1.4 ~~1.2~~ 1.2 1 0.1

10X T Mg

10 8.67 7.33 6 4.67 8 6.67 5.33

10X - Mg

0 4.33 2.67 4 5.33 2.67 5.33 8 10.67 12 13.33 14

~~50~~ red - (120  $\mu$ l)9.6  $\mu$ l 5/4 dye 10.4 H<sub>2</sub>O(H<sub>2</sub>O) white

12

108

white

12 50% gly, 108 H<sub>2</sub>O2X Emix (275  $\mu$ l)5.5 Tag (50  $\mu$ l), 11  $\mu$ l ea pm $\lambda$  - 27.5  $\mu$ l, dATP's 5.5 27.55  $\mu$ l dATP

209.5

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

L. J. J.

Date

Invented by

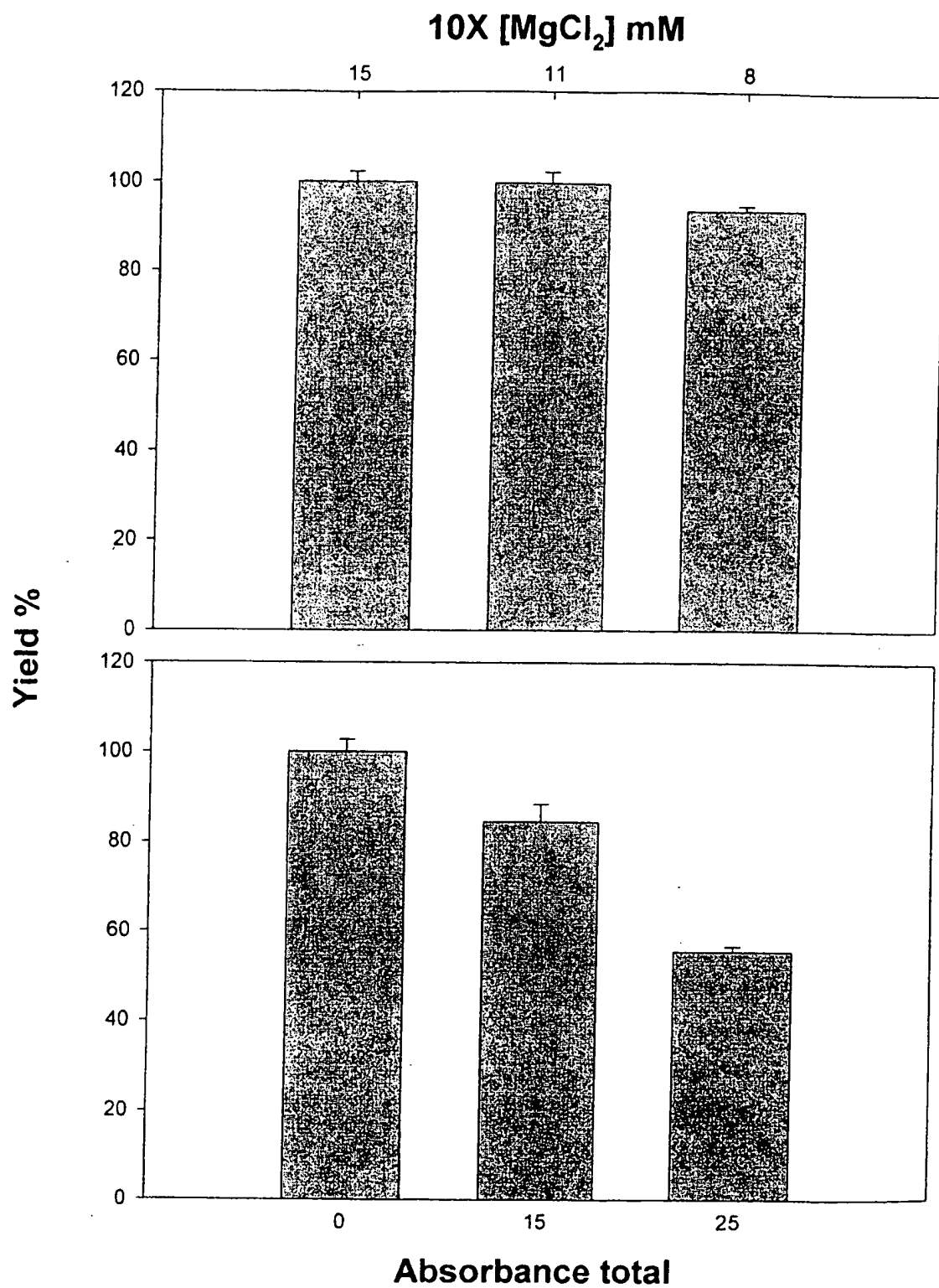
L. J. J.

Date

Recorded by

*David Red*

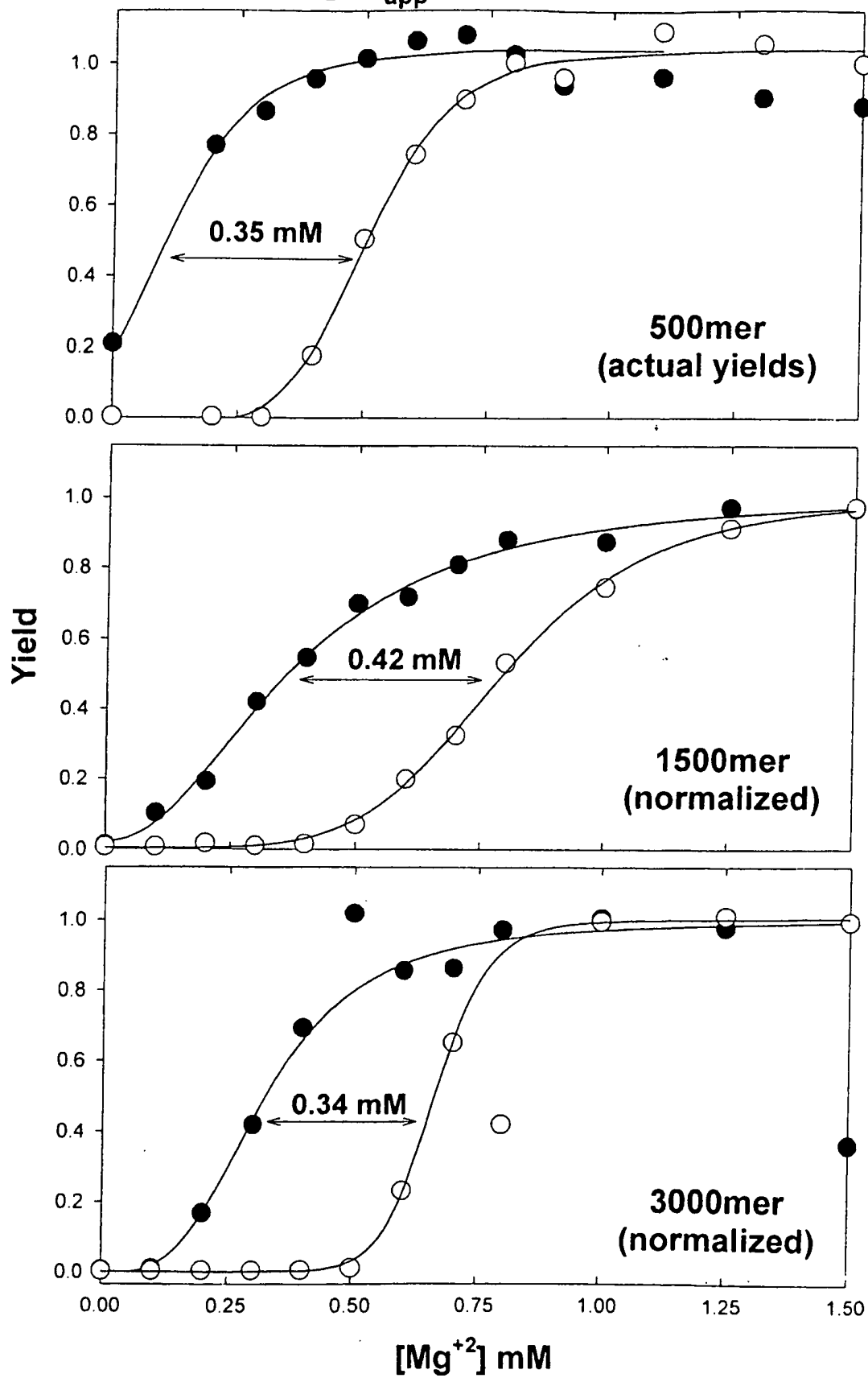
# Yield of 500mer and 3000mer for RedTaq compensated for $[Mg^{+2}]$



USER: 1 ID: BONEHEAD      PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N    RS232: N  
 H#: 0 AOC: N BCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CPM. UNKNOWN REPLICATES: 1    NORM FACTOR: 0 1.00000  
 HALF LIFE (DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	567820.00	1.19	0.05	0.22	
2	**	2	592040.00	1.16	0.05	0.61	
3	**	3	535880.00	1.22	0.05	0.98	
4	**	4	579840.00	1.17	0.05	1.36	
5	**	5	580220.00	1.17	0.05	1.73	
6	**	6	548380.00	1.21	0.05	2.11	
7	**	7	594480.00	1.16	0.05	2.48	
8	**	8	563900.00	1.19	0.05	2.86	
9	**	9	546180.00	1.21	0.05	3.23	
10	**	10	266920.00	1.73	0.05	3.61	
11	**	11	268040.00	1.73	0.05	3.97	
12	**	12	245600.00	1.80	0.05	4.33	
13	**	1	585680.00	1.17	0.05	4.78	
14	**	2	480660.00	1.29	0.05	5.16	
15	**	3	321860.00	1.58	0.05	5.53	
16	**	4	613700.00	1.14	0.05	5.90	
17	**	5	526320.00	1.23	0.05	6.27	
18	**	6	336100.00	1.54	0.05	6.65	
19	**	7	585660.00	1.17	0.05	7.02	
20	**	8	497640.00	1.27	0.05	7.40	
21	**	9	329180.00	1.56	0.05	7.77	
22	**	10	421780.00	1.38	0.05	8.14	
23	**	11	358580.00	1.49	0.05	8.52	
24	**	12	221420.00	1.90	0.05	8.88	

Apparent  $[Mg^{2+}]$  of red taq.  
 $[Mg^{+2}]_{app} = 0.37 \pm 0.04 \text{ mM}$



*Handwritten signature*



1500 bp  $\lambda$

SER: 1 ID: BONEHEAD PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
 H#: 0 AQC: N QCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000  
 HALF LIFE(DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	10xEmg3	ERR
1	**	1	57715.00	1.86	0.20	0.38	15	—
2	**	2	57755.00	1.86	0.20	0.90	12.5	
3	**	3	51535.00	1.96	0.20	1.42	10	
4	**	4	52230.00	1.96	0.20	1.95	8	
5	**	5	48012.00	1.83	0.25	2.52	7	Red
6	**	6	42568.00	1.94	0.25	3.10	6	
7	**	7	41480.00	1.96	0.25	3.67	5	
8	**	8	32380.00	1.88	0.35	4.35	4	
9	**	9	24697.78	1.89	0.45	5.13	3	
10	**	10	11444.44	1.97	0.90	6.37	2	
11	**	11	6144.00	2.55	1.00	7.71	1	
12	**	12	735.00	7.38	1.00	9.04	0	—
13	**	1	71333.33	1.93	0.15	9.59	15	
14	**	2	66820.00	2.00	0.15	10.07	12.5	
15	**	3	54565.00	1.91	0.20	10.58	10	
16	**	4	38780.00	1.85	0.30	11.21	8	
17	**	5	23611.11	1.94	0.45	11.99	7	
18	**	6	14545.71	1.98	0.70	13.02	6	White
19	**	7	5161.00	2.78	1.00	14.35	5	
20	**	8	1119.00	5.98	1.00	15.68	4	
21	**	9	756.00	7.27	1.00	17.02	3	
22	**	10	1225.00	5.71	1.00	18.36	2	
23	**	11	560.00	8.45	1.00	19.70	1	
24	**	12	546.00	8.56	1.00	21.04	0	—

5000 by 2

ID: BONEHEAD PRESET TIME: 1.00  
REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
O AQO: N QCF: N RCN: N  
CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000  
HALF LIFE (DAYS): N

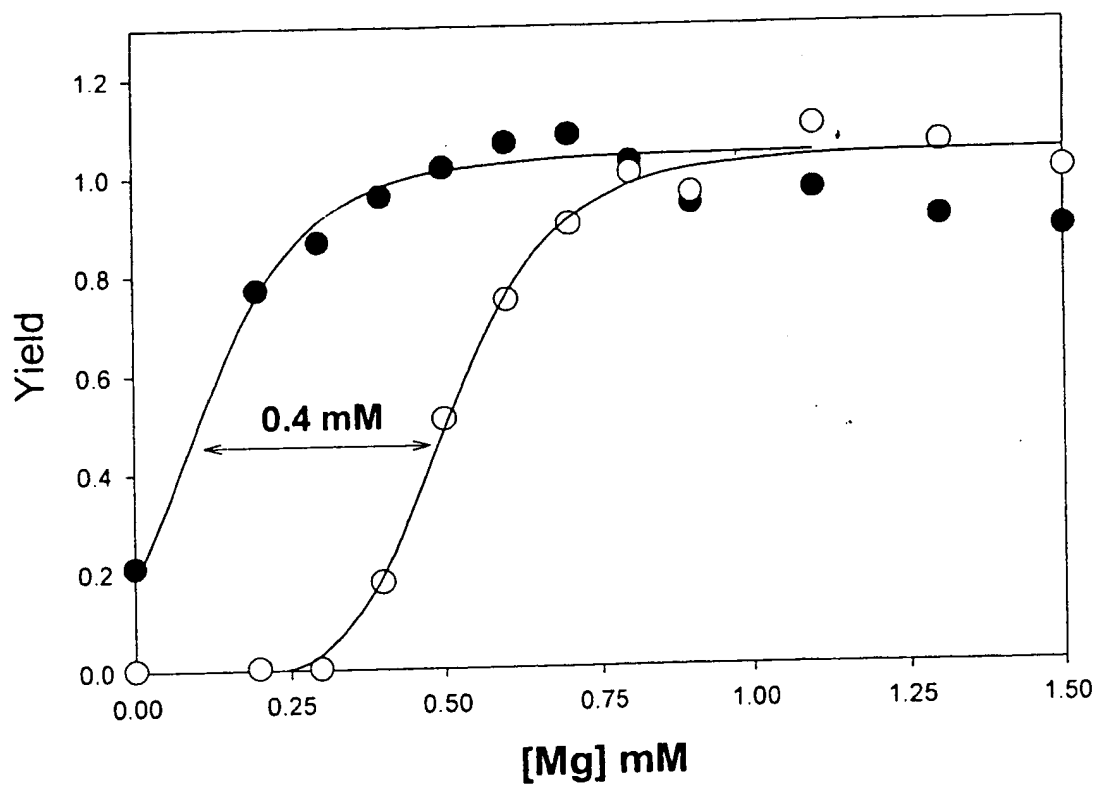
SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	27832.50	1.90	0.40	0.58	15
2	**	2	75666.66	1.88	0.15	1.06	12.5
3	**	3	77986.66	1.85	0.15	1.53	10
4	**	4	75440.00	1.88	0.15	2.00	8
5	**	5	67020.00	1.99	0.15	2.47	7
6	**	6	66455.00	1.73	0.20	2.99	6
7	**	7	79033.33	1.84	0.15	3.47	5
8	**	8	53350.00	1.93	0.20	3.99	4
9	**	9	32408.57	1.88	0.35	4.67	3
10	**	10	13086.25	1.95	0.80	5.80	2
11	**	11	861.00	6.82	1.00	7.13	1
12	**	12	497.00	8.97	1.00	8.47	0
13	**	1	86740.00	1.75	0.15	9.00	15
14	**	2	88366.66	1.74	0.15	9.48	12.5
15	**	3	87173.33	1.73	0.15	9.95	10
16	**	4	36680.00	1.91	0.30	10.58	8
17	**	5	57195.00	1.87	0.20	11.10	7
18	**	6	20180.00	1.99	0.50	11.93	6
19	**	7	999.00	6.33	1.00	13.27	5
20	**	8	440.00	30.15	0.10	13.68	4
21	**	9	420.00	30.86	0.10	14.13	3
22	**	10	470.00	29.17	0.10	14.55	2
23	**	11	335.00	24.43	0.20	15.08	1
24	**	12	300.00	29.81	0.15	15.55	0

10X [mg]

red

white

Apparent  $[Mg^{2+}]$  of red taq.  
Red curve is white curve displaced by 0.4 mM



ID: BONEHEAD      PRESET TIME: 1.00  
 REPEAT: 1 CYCLE REPEAT: 1 SCR: N      RS232: N  
 ADC: N QCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CPM. UNKNOWN REPLICATES: 1      NORM FACTOR: 0 1.00000  
 ALF LIFE(DAYS): N

AM	FDS	CH	CPM	2SIG%	TIME	EL TIME	[Mg] mM	ERR
1	**	1	362180.00	1.49	0.05	0.22	1.5	
2	**	2	372200.00	1.47	0.05	0.60	1.3	
3	**	3	396460.00	1.42	0.05	0.97	1.1	
4	**	4	385060.00	1.44	0.05	1.35	0.9	
5	**	5	420960.00	1.38	0.05	1.72	0.8	
6	**	6	443840.00	1.34	0.05	2.10	0.7	
7	**	7	437640.00	1.35	0.05	2.47	0.6	
8	**	8	417680.00	1.38	0.05	2.85	0.5	
9	**	9	393960.00	1.43	0.05	3.22	0.4	
10	**	10	355980.00	1.50	0.05	3.60	0.3	
11	**	11	316460.00	1.59	0.05	3.97	0.2	
12	**	12	86620.00	1.75	0.15	4.45	0	
13	**	1	411420.00	1.39	0.05	4.93	1.5	
14	**	2	434200.00	1.36	0.05	5.30	1.3	
15	**	3	449840.00	1.33	0.05	5.68	1.1	
16	**	4	394500.00	1.42	0.05	6.05	0.9	
17	**	5	411460.00	1.39	0.05	6.43	0.8	
18	**	6	369000.00	1.47	0.05	6.80	0.7	
19	**	7	306420.00	1.62	0.05	7.18	0.6	
20	**	8	208220.00	1.96	0.05	7.53	0.5	
21	**	9	72633.33	1.91	0.15	8.01	0.4	
22	**	10	1056.00	12.31	0.25	8.58	0.3	
23	**	11	1575.00	11.27	0.20	9.11	0.2	
24	**	12	1208.33	7.43	0.60	10.06	0	

Page No. \_\_\_\_\_

## PCR yields

Compare red @ 1  $\mu$ /ul with  
white @ 5  $\mu$ /ul

1. 3X red/buffer

agg = 45, buffer = 3X

10X Buffer = 11.11 mM  $MgCl_2$

10X (200  $\mu$ l) = 153.33 10X + Mg  
40.67 10X - Mg

3X- (200  $\mu$ l) 24  $\mu$ l 5/4,

48.88  $\mu$ l 10X + Mg

11.11  $\mu$ l 10X - Mg

116  $H_2O$

3X White Buffer 60  $\mu$ l 10X + Mg, 190  $H_2O$

3X PMR 2-6 50  $\mu$ l ea 3  $\mu$ l pmr  
47  $\mu$ l  $H_2O$

3X Enz - 275  $\mu$ l  $\lambda$  PMR.1 - 16.5  $\mu$ l

$\lambda$  ~~275~~  $\mu$ l 13.5  $\mu$ l

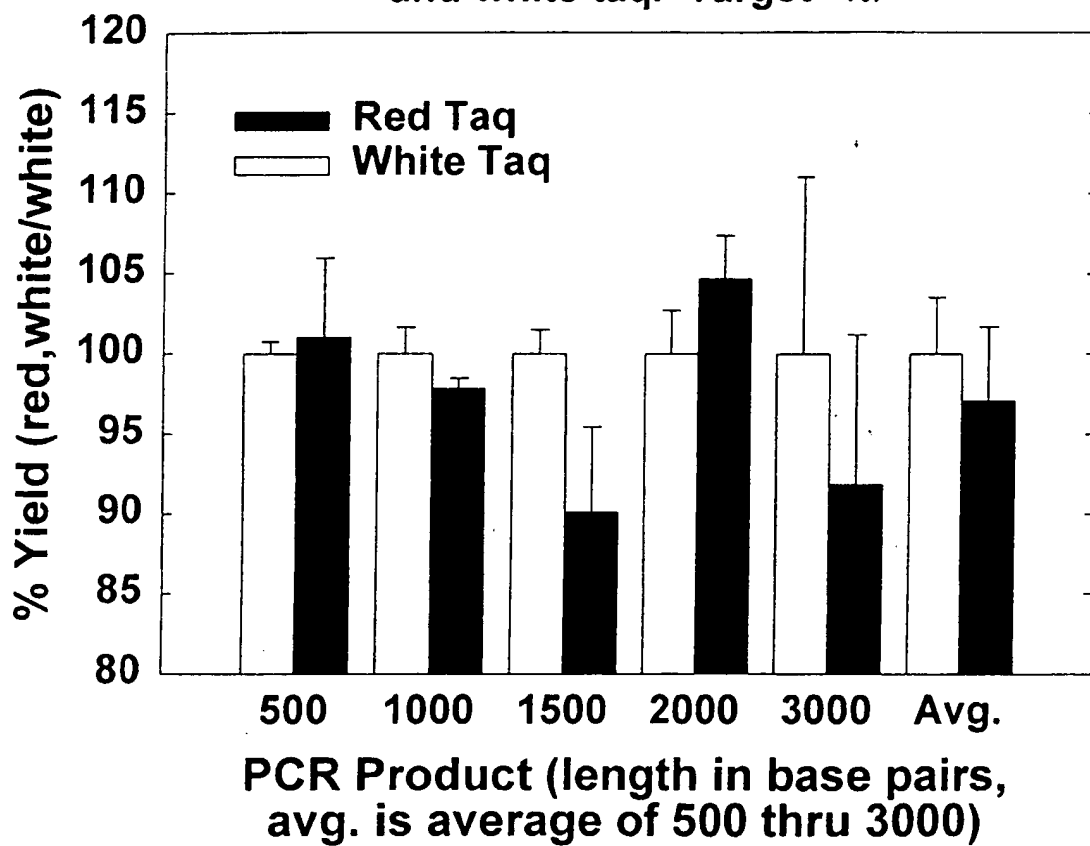
10 mM dNTP's 8.25  $\mu$ l, Tag 8.25

$\alpha^{32}P$  dCTP 5  $\mu$ l, 228.5  $H_2O$

To Page No. \_\_\_\_\_

*Erin Reed*

PCR product yields using red  
and white taq. Target =  $\lambda$ .



yield

ER: 1 ID: BONEHEAD      PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N      RS232: N  
 H#: 0 AGC: N DCF: N REM: N  
 CHANNEL I-LL: 0 UL: 1000 2SIDMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CPM. UNKNOWN REPLICATES: 1      NORM FACTOR: 0 1.00000  
 HALF LIFE (DAYS): N

SAM	PUS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	924380.00	0.93	0.05	0.23 R	-
2	**	2	956780.00	0.91	0.05	0.62 W	500
3	**	3	1019260.00	0.89	0.05	1.00 R	-
4	**	4	1031520.00	0.88	0.05	1.38 W	1000
5	**	5	780480.00	1.01	0.05	1.77 R	-
6	**	6	820460.00	0.99	0.05	2.15 W	1500
7	**	7	381360.00	1.45	0.05	2.53 R	-
8	**	8	357640.00	1.50	0.05	2.90 W	2000
9	**	9	1012000.00	0.89	0.05	3.28 R	-
10	**	10	1101220.00	0.84	0.05	3.67 W	3000
13	**	1	969840.00	0.91	0.05	4.15	.
14	**	2	958300.00	0.91	0.05	4.53	.
15	**	3	1023520.00	0.88	0.05	4.92	.
16	**	4	1066120.00	0.87	0.05	5.30	.
17	**	5	767360.00	1.02	0.05	5.68	.
18	**	6	844480.00	0.97	0.05	6.07	.
19	**	7	377740.00	1.46	0.05	6.44	.
20	**	8	377360.00	1.46	0.05	6.82	.
21	**	9	1047740.00	0.87	0.05	7.20	.
22	**	10	925920.00	0.93	0.05	7.58	.

10x Red = 11 m m Mg

SER: 1 ID: BONEHEAD PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232: N  
 HH: 0 ABC: N DCF: N RCM: N  
 CHANNEL 1-LL: 0 UL: 1000 2SIGMA: 2.00 PKG SUB: 0.00 PKG 2SIG: 0.00 LSR: 0  
 DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 0 1.00000  
 HALF LIFE (DAYS): N

SAM	POS	CH	CPM	2SIG%	TIME	EL TIME	ERR
1	**	1	1019620.00	0.89	0.05	0.23	
2	**	2	969680.00	0.91	0.05	0.62	
3	**	3	1032600.00	0.88	0.05	1.01	
4	**	4	1045260.00	0.87	0.05	1.39	
5	**	5	697540.00	1.07	0.05	1.77	
6	**	6	826540.00	0.98	0.05	2.15	
7	**	7	396480.00	1.42	0.05	2.52	
8	**	8	368860.00	1.47	0.05	2.90	
9	**	9	860500.00	0.96	0.05	3.28	
10	**	10	1112760.00	0.85	0.05	3.67	



Originated

#### Red Taq preparation.

1. **Prepare Mg Acid Red 1 and Mg Acid Violet solutions.** Dissolve dyes in *Taq* dilution buffer at approximately 10 mg/ml, 0.2  $\mu$ m filter. Note: dissolution of Acid Red 1 generally requires heating in a hot (90°C or above) water bath.
2. **Measure solution absorbances.** To a tared 15 ml conical tube add approximately 10 ml of water, record weight of water ( $w$ ). Add approximately 10  $\mu$ l of dye solution and record weight ( $d$ ). Cap and mix. Calculate dilution factors ( $f$ ).  $f = 1.1423 \times d / (d - w)$ . 1.1423 is *Taq* dilution buffer density. Measure absorbance spectrum for each diluted dye solution. Record absorbance ( $A_{AR1}$  and  $A_{AV2}$  for acid red 1 and acid violet 5 respectively) of each solution at their respective  $\lambda_{max}$ . Acid Red 1  $\approx$  532 nm, Acid Violet 5  $\approx$  527 nm.
3. **Calculate absorbance of dye concentrates.**  $A_c = A_d \times f$ ,  $A_c$  is concentrate absorbance in absorbance units per ml.
4. **Calculate Red *Taq* dilution volumes.** The final solution will have *Taq* at 1 u/ $\mu$ l, Acid Red 1 at 240 abs. units per ml and Acid Violet 5 at 60 abs. units per ml. For  $v$  ml of solution the final weight ( $wf$ ) will be  $1.1423 \times v$ . The solution will contain:  $(240 / A_{AR1}) \times wf$  of Acid Red 1,  $(60 / A_{AV2}) \times wf$  of Acid Violet 5,  $0.8 \times wf - ((240 / A_{AR1}) \times wf + (60 / A_{AV2}) \times wf)$  of *Taq* dilution buffer and  $0.2 \times wf$  of *Taq*.
5. **Prepare solution.** The solution is prepared in a sterile environment (i.e. hood) by weighing the components into a tared sterile container. The container will depend on the size of the lot being produced. If possible, solutions should be added to a capable bottle, i.e. conical tubes, Nalgene bottles etc. Thus, on a balance in a laminar flow hood, carefully weigh each component into the tared container. Record the weight of each addition. Retare after each addition. Solution additions can generally be within  $\pm 1\%$ . Thoroughly mix after the last component is added.
6. **Perform Suitability assay.** PCR products (lambda and 500mer primers, QC SOP ) using Red *Taq* and 10X Red *Taq* buffer should be indistinguishable from *Taq* (D1806) and 10X PCR buffer.